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VOLATILE COMPOUNDS OF IMPORTANCE IN THE AROMA OF CULTIVATED MUSHROOMS *AGARICUS BISPORUS* AT DIFFERENT CONDITIONS OF CULTIVATION

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Aroma in samples of two varieties of *Agaricus bisporus*, *i.e.* KORONA 7 and EUROMYCEL 12, was analysed in terms of contents of eight carbon atom compounds (1-octen-3-ol, 3-octanone, 3-octanol, 2-octen-1-ol). Carpophores were harvested in 4 flushes according to their cap sizes. Quantitatively the dominant compound in all samples was 1-octen-3-ol, found in the highest concentrations in the 1st flush of yielding. The analysis of variance for factorial experiments, performed for both cultivars in one cultivation cycle – 2001/2002 and for one variety – KORONA 7 in two cycles, showed the effect of mushroom variety, flush of yielding and carpophores size on contents of dry matter and aromatic volatiles. Carpophores of a smaller cap diameter were characterised by a higher dry matter content and usually also higher contents of aromatic volatiles. In contrast, the effect of flush of yielding was different: the highest content of dry matter was recorded in the 4th flush, while that of aromatic compounds in the 1st flush.

INTRODUCTION

The production of *Agaricus bisporus* accounts for most of world production of cultivated mushrooms [Kües & Liu, 2000; Ragunathan & Swaminathan, 2003]. Their commercial value is very high mostly due to their flavour. Mushrooms have long been used as food or food-flavoring material because of their unique and subtle flavour [Mau *et al.,* 1992]. The mushroom volatiles have been the subject of many investigations and aroma compounds responsible for the characteristic flavour of certain mushroom species have been well documented [Wąsowicz, 1974; Maga, 1984; Chen & Wu, 1984; Chen *et al.*, 1984; Fischer & Grosch, 1987; Mau *et al.*, 1992; 1997; Assaf *et al.*, 1997, Lizarraga-Guerra *et al.*, 1997, Venkateshwarlu *et al.*, 1999]. The main odorants of mushroom aroma are eight carbon atom (C8) compounds; the most important of which is 1-octen-3-ol. It is found in two optically active forms [Mosandl *et al.*, 1986, Bauer *et al.*, 1990; Chambers *et al*, 1998]. 1-Octen-3-ol is a characteristic aroma compound of mushrooms, occurring in most of them in the amounts ranging *ca*. 1–3 mg/100 g of fresh matter [Mau *et al*., 1992; Mau & Hwang, 1997; Venkateshwarlu *et al*., 1999], in some species in smaller quantities, but in every case with a specific enantiomeric ratio and optical purity of the minus form, in *Agaricus bisporus* even over 99% [Zawirska-Wojtasiak, 2004]. The profile of flavour compounds varies with species and variety and can also be influenced by culture conditions [Venkateshwarlu *et al*., 1999]. Cultivation conditions and varieties may also affect other mushroom properties, *i.a.* dry matter and morphological features of carpophores. Dry matter is one of the very important markers of mushroom processability. Carpophores which are characterised by high dry matter content lose less of their volume during blanching. According to literature sources, carpophores of *Agaricus bisporus* differ significantly in their dry matter content. In *Agaricus bisporus* it was estimated to range from 6 to 12% [Oei, 2003] and from 9.5 to 11.7.% [Crisan & Sands; 1978]. In several experiments carried out in Poland under the conditions of intensive cultivation, the observed level of dry matter content ranged from 6.5 to 8.5% [Kiśluk *et al.,* 1997; Siwulski *et al*., 2000].

The aim of this study was to determine the concentration of selected major volatiles and dry matter content of carpophores in samples of experimentally grown *Agaricus bisporus* in relation to variety, flush of yielding and size of caps.

MATERIALS AND METHODS

Mushrooms and chemicals. Samples of fresh *Agaricus bisporus* were taken from an experimental cultivation plot at the Department of Vegetable Crops, the August Cieszkowski Agricultural University of Poznań, in 2001, 2002, 2003. Two varieties of mushrooms were analysed: KORONA 7 and EUROMYCEL 12. The cultivations were conducted in the winter-spring seasons. The experimental culture was established in a mushroom growing room with full controlled cultivation conditions in Kostrzyn Wielkopolski. The substrate was prepared from wheat straw and chicken dung. The culture was carried out in accordance with the respective recommendations for modern garden mushroom culture. The

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experiment was designed in four replications. Three culture cycles were applied. The mushrooms were harvested in the button stage, at four flushes of crop (I, II, II and IV). The carpophores of the first flush were picked up after 2–3 days. The carpophores of the next flush appeared after 5–6 days. Mushrooms were sorted due to the size of caps into two grades of diameter, 3.0–3.5 and 2.0–2.5 cm.

Standards of 1-octen-3-ol and other chemicals used for the identification of volatile compounds were purchased from Sigma Chemical Co. and the other reagents, such as pentadecane, ethyl ether and pentane, were of analytical grade for GC.

Isolation of volatiles by microdistillation-extraction procedure. The volatiles from mushrooms were isolated by the micro-steam distillation extraction-procedure in a Likens-Nickerson apparatus [Bouseta & Collin, 1995], with ether-pentane (1:1 v/v) as the extraction solvent. Distillation was carried out in a Chrompac microextractor. The amount of 75 g fresh mushrooms was used for isolation. Pentadecane as the internal standard was added (0.6 mg) before distillation. Fresh mushrooms were cut into pieces and homogenized with 150 mL of distilled water for 5 min. The homogenate was put into 250 mL flask, left for 15 min to maximize the enzymatic production of flavour [Venkateshwarlu *et al.*, 1999] and subjected to simultaneous distillation and solvent extraction for 2 h without reduced pressure. The cold finger was connected to a container with ice/water and cooling pump. The flavor extract was dried over anhydrous sodium sulphate and concentrated to 0.1 mL, blowing down with nitrogen at room temperature. The recovery of 1-octen-3-ol was calculated using test mixture of this compound. Comparing peak area ratios of the component and internal standard in steam distillate with those in reference gave recovery which amounted to 76 % for 1-octen-3-ol added.

Gas chromatography analysis. A Hewlett-Packard HP 6890 gas chromatograph with a split/splitless injector and an FID detector was used for the analyses. Compounds were separated using an Innovax capillary column (Hewlett-Packard, 30 m × 0.25 mm × 0.25 μm). For the identification procedure the Kovats' retention indexes – RI [Ševčik, 1977] were estimated. The identity of separated compounds was confirmed on a Hewlett-Packard HP 5890 II gas chromatograph with MDN-5 column (Supelco Inc., Bellefonte, PA, 30 m × 0.25 mm × 0.25 μm) coupled to an HP 5971MSD quadrupole mass spectrometer. Analysis parameters on the Innowax column were as follows: initial temp. 60°C, then 30°C/min to 200°C. The flow of hydrogen, used as a carrier gas, was 1.6 mL/min. A 1-μL portion of the prepared sample was injected in a split mode 1:100. The fuel gases there were air and hydrogen at the flow rates respectively 300 mL/min and 30 mL/min, while nitrogen was used as make-up at a flow rate of 20 mL/min. The temperature of an injector was set at 230°C, and that of a detector at 260°C. The concentrations of volatiles were calculated on the basis of the known amount of the internal standard added to the sample prior to distillation.

Estimation of dry matter content. Content of carpophores dry matter was determined in 500-g samples. Carpophores were cut into slices approx. 2 mm thick and predried at 40°C for 4 h and then forced dried at 105°C to a constant weight.

Statistical analysis. Statistical analysis of data was performed using the analysis of variance for factorial experiments. Means were compared using the Newman-Keuls test at α=0.05.

RESULTS AND DISCUSSION

The resolution of volatiles in mushroom distillates was obtained using an Innowax column. The calculated retention indexes for the separated compounds were compared with those for the standards. The identified compounds included hexanal (RI 1110), 3-methyl butanol (RI 1201), 3-octanone (RI 1268), hexanol (RI 1354), 3-octanol (RI 1489), 1-octen-3-ol (RI 1451), 2-octen-1-ol (RI 1703), also mentioned by other authors [Venkateshwarlu *et al.*, 1999; Maga, 1984]. Similar indexes were obtained by Venkateshwarlu *et al.* [1999] on a PE-wax column and by Fischer & Grosch [1987] on Supelcowax 10. Four main eight carbon atom volatiles were taken into consideration for quantitative analysis: 3-octanone, 3-octanol and 1-octen-3-ol and 2-octen-1-ol. Table 1 presents the concentrations of these compounds in samples

TABLE 1. The concentration of main volatiles (mg/100 g fresh matter) in the samples of variety KORONA 7 (2000/2001).

Flush	3-Octanone*	3-Octanol**	1-Octen-3-ol*	2-Octen-1-ol**
		cap diameter	2.0–2.5 cm	
I	0.49	0.14	5.20	0.72
II	0.46	0.05	1.55	0.12
III	0.76	0.06	1.33	0.06
IV	0.42	0.06	1.18	0.12
		cap diameter	3.0–3.5 cm	
I	0.72	0.22	5.08	0.99
II	0.73	0.05	1.96	0.14
III	0.47	0.05	1.14	0.18
IV	0.18	0.06	0.82	0.09

coefficient of variation: *<5%, ** <7%

TABLE 2. The concentration of main volatiles (mg/100 g fresh matter) n the samples of variety KORONA 7 (2001/2002).

Flush	3-Octanone*	3-Octanol**	1-Octen-3-ol*	2-Octen-1-ol**
		cap diameter	2.0–2.5 cm	
I	1.63	0.96	2.11	0.30
II	0.40	0.04	1.03	0.12
III	0.39	0.07	0.77	0.07
IV	0.26	0.06	0.77	0.06
		cap diameter	3.0–3.5 cm	
I	1.11	0.35	1.02	0.23
II	0.39	0.06	0.81	0.11
III	0.18	0.04	0.76	0.07
IV	0.16	0.01	0.58	0.07

coefficient of variation: *<5%, ** <7%

TABLE 3. The concentration of main volatiles (mg/100 g fresh matter) in the samples of variety EUROMYCEL 12 (2001/2002).

Flush	3-Octanone*	3-Octanol**	1-Octen-3-ol*	2-Octen-1-ol**
		cap diameter	2.0–2.5 cm	
I	1.48	0.44	1.67	0.13
II	1.04	0.22	1.26	0.08
III	0.16	0.03	0.38	0.07
IV	0.14	0.02	0.40	0.06
		cap diameter	3.0–3.5 cm	
I	1.07	0.18	1.06	0.11
II	0.42	0.09	1.16	0.09
III	0.11	0.02	0.25	0.02
IV	0.23	0.04	0.46	0.06

coefficient of variation: *<5%, ** <7%

TABLE 4. The concentration of main volatiles (mg/100 g fresh matter) in the samples of variety EUROMYCEL 12 (2002/2003).

Flush	3-Octanone*	3-Octanol**	1-Octen-3-ol*	2-Octen-1-ol**
		cap diameter	2.0–2.5 cm	
I	0.67	0.28	2.30	0.23
II	0.11	0.13	1.31	0.11
III	0.16	0.10	1.01	0.15
IV	0.04	0.11	0.84	0.10
		cap diameter	3.0–3.5 cm	
I	1.06	0.24	1.50	0.13
II	0.19	0.12	1.05	0.12
III	0.12	0.04	0.60	0.03
IV	0.13	0.03	0.63	0.04

coefficient of variation: *<5%, ** <7%

of variety KORONA 7 cultivated in the season 2000/2001 for two sizes of carpophores caps, respectively, while Tables 2 gives concentrations in samples of the same variety cultivated in the season 2001/2002. Table 3 presents concentrations for variety EUROMYCEL 12 cultivated in the season 2001/2002 and Tables 4 those for EUROMYCEL 12 in the season 2002/2003.

In the majority of samples 1-octen-3-ol occurred in the highest amounts (from about 1 to 5 mg/100 g of fresh matter). This compound was also mentioned by other authors [Maga, 1984; Mau *et al*., 1992; Fisher & Grosh, 1987] as the main odorant of *Agaricus bisporus*. Its concentration was higher in smaller caps (2.0–2.5 cm) than in the bigger ones (3.0–3.5 cm) in all measured samples, but this difference was only rarely statistically significant. In all the experiments with both varieties the highest volatile concentrations, particularly of 1-octen-3-ol, were recorded in the first flush of crops. It was very distinct in the variety KORONA; for example in the first flush 5.20 mg/100 g, while in the second only 1.55 mg/100 g (Table 1). On the basis of statistical interpretation by the Keuls-Newman test done for volatiles of both varieties occurring in one season 2001/2002 (Tables 5 and 6) and for volatiles for one variety KORONA 7 occurring in two seasons (Tables 7 and 8) it can be stated that the amounts of volatiles in mushrooms were affected by the variety, flush of crop and size of cap. The concentration of total volatiles, as well as that of 1-octen-3-ol was significantly higher in variety KORONA 7 when comparing varieties in one cycle of 2001/2002. It was also higher in the samples with a smaller cap diameter, but significantly only in variety KORONA 7. The influence of the variety on the flavour profile of various plants was pointed out in literature [Zawirska-Wojtasiak *et al.,* 1998], also in the case of mushrooms [Venkateshwarlu *et al.*, 1999]. The concentration of total volatiles and 1-octen-3-ol was also higher in the 1st and 2nd flushes of crops than

TABLE 5. Total flavour compounds (mg/100 g fresh matter) – cycle 2001/2002.

Variety	Cap diameter (cm)	Flush				Mean value for cap diameter	Mean value for variety
		I	II	III	IV		
KORONA 7	2.0–2.5	4.45	2.08	1.37	1.24	2.29^{b*}	1.99^{b*}
	3.0–3.5	2.86	1.74	1.20	0.99	1.69^{a}	
EUROMYCEL 12	2.0–2.5	3.29	2.49	0.64	0.65	1.77^{a}	1.61^{a}
	3.0–3.5	2.78	1.78	0.41	0.85	1.45^{a}	
Mean value for flush		3.35^{C**}	2.02^{B}	0.91^{A}	0.93^{A}		

*values in columns marked with the same letters are not significantly different at α=0.05; ** values in lines marked with the same letters are not significantly different at α=0.05

TABLE 6. Concentration of 1-octen-3-ol (mg/100 g fresh matter) – cycle 2001/2002.

Variety	Cap diameter (cm)	Flush				Mean value for cap diameter	Mean value for variety
		I	II	III	IV		
KORONA 7	2.0–2.5	2.11	1.03	0.77	0.77	1.17^{b*}	0.98^{b*}
	3.0–3.5	1.03	0.81	0.76	0.58	0.79^{a}	
EUROMYCEL 12	2.0–2.5	1.67	1.26	0.38	0.41	0.93^{a}	0.83^{a}
	3.0–3.5	1.06	1.16	0.25	0.46	0.74^{a}	
Mean value for flush		1.47^{C**}	1.06^{B}	0.54^{A}	0.55^{A}		

*values in columns marked with the same letters are not significantly different at α=0.05; ** values in lines marked with the same letters are not significantly different at α=0.05

TABLE 7. Total flavour compounds (mg/100 g fresh matter) – variety KORONA 7.

Cycle	Cap diameter (cm)	Flush				Mean value for cap diameter	Mean value for cycle
		I	II	III	IV		
2000/2001	2.0–2.5	6.56	2.40	2.11	1.71	3.19^{c*}	3.14^{b*}
	3.0–3.5	6.41	2.87	1.90	1.19	3.09^{c}	
2001/2002	2.0–2.5	4.45	2.08	1.37	1.24	2.29^{b}	1.99^{a}
	3.0–3.5	2.86	1.74	1.20	0.99	1.70^{a}	
Mean value for flush		5.07^{C**}	2.27^{B}	1.65^{A}	1.28^{A}		

*values in columns marked with the same letters are not significantly different at α=0.05; ** values in lines marked with the same letters are not significantly different at α=0.05

TABLE 8. Concentration of 1-octen-3-ol (mg/100 g fresh matter) – variety KORONA 7.

Cycle	Cap diameter (cm)	Flush				Mean value for cap diameter	Mean value for cycle
		I	II	III	IV		
2000/2001	2.0–2.5	5.12	1.55	1.33	1.18	2.31^{c*}	2.28^{b*}
	3.0–3.5	5.08	1.96	1.15	0.82	2.25^{c}	
2001/2002	2.0–2.5	2.11	1.03	0.77	0.77	1.17^{b}	0.98^{a}
	3.0–3.5	1.03	0.81	0.76	0.58	0.79^{a}	
Mean value for flush		3.35^{C**}	1.34^{B}	1.00^{A}	0.84^{A}		

*values in columns marked with the same letters are not significantly different at α=0.05; ** values in lines marked with the same letters are not significantly different at α=0.05

TABLE 9. Dry matter content in two varieties of *Agaricus bisporus* (%).

Cycle	Cap diameter (cm)	Flush				Mean value for cap diameter	Mean value for cycle
		I	II	III	IV		
KORONA 7	2.0–2.5	8.5	8.4	8.5	8.8	8.5^{c*}	8.4^{b*}
	3.0–3.5	8.2	8.3	8.3	8.5	8.3^{b}	
EUROMYCEL 12	2.0–2.5	7.9	7.8	8.0	8.2	8.4^{b}	7.8^{a}
	3.0–3.5	7.7	7.6	7.6	7.9	7.7^{a}	
Mean value for flush		8.0^{A**}	8.0^{A}	8.1^{A}	8.3^{B}		

*values in columns marked with the same letters are not significantly different at α=0.05; ** values in lines marked with the same letters are not significantly different at α=0.05

TABLE 10. Dry matter content in variety KORONA 7 (%).

Cycle	Cap diameter (cm)	Flush				Mean value for cap diameter	Mean value for cycle
		I	II	III	IV		
2000/2001	2.0–2.5	8.5	8.4	8.5	8.8	8.5^{c*}	8.4^{b*}
	3.0–3.5	8.2	8.3	8.3	8.5	8.3^{b}	
2001/2002	2.0–2.5	8.2	8.2	8.3	8.4	8.3^{b}	8.2^{a}
	3.0–3.5	8.0	7.9	8.1	8.3	8.0^{a}	
Mean value for flush		8.2^{A**}	8.2^{A}	8.3^{A}	8.5^{B}		

*values in columns marked with the same letters are not significantly different at α=0.05; ** values in lines marked with the same letters are not significantly different at α=0.05

in the 3rd and 4th – in the 1st flush the concentration was the highest. The last observation was also stated when comparing one variety (KORONA 7) in two cycles (2000/2001 and 2001/2002). The concentration of volatiles was higher in smaller caps in all the samples of this variety, but significantly only in the cycle of 2001/2002. The cycles differed significantly – the concentration of volatiles was higher in the cycle of 2000/2001.

Similar statistical interpretation was done for dry matter content (Tables 9 and 10) and it was also stated that varieties, flush of crop and size of carpophores influenced dry matter content.

Variety KORONA 7 was characterised by carpophores with a higher dry matter content than EUROMYCEL 12. The difference of 0.6% between varieties was estimated. Differences in dry matter contents of *Agaricus bisporus* carpo-

phores were also reported by Siwulski *et al*. [2000], Kiśluk *et al*. [1997] and Sobieralski & Siwulski [1993].

Contents of carpophores coming from the first three flushes were, with few exceptions, uniform and no statistically significant differences ($p>0.005$) were shown between them. However, it was found that carpophores from the fourth flush contained significantly more dry matter in comparison to carpophores of the other flushes. It was observed for the both tested varieties of mushrooms. Differences in the quality of carpophores harvested in the same development stage from different flushes were previously shown by Burton & Noble [1993].

Studies showed that carpophores with a cap diameter of 2.0-2.5 cm were characterised by a significantly higher dry matter content in comparison to carpophores with a cap diameter of 3.3–3.5 cm. The difference shown was observed in all yielding flushes and was characteristic for both varieties analysed.

Differences were observed in dry matter contents of carpophores coming from different cultivation cycles (Table 10). Carpophores from the cultivation cycle 2000/2001 were characterised by a higher dry matter content than the carpophores coming from the cycle 2001/2002. In turn, the effect of the other experimental factors, *i.e.* cap diameter and yielding flush, was identical as in the previously discussed experiment.

CONCLUSIONS

In conclusion, it may be stated that all discussed factors the, *i.e.* cultivation cycle, variety, flush and carpophores size, may affect the dry matter content and odorant concentrations in edible mushroom. The effect of these factors on the analysed characters was similar, except for yielding flush. In case when mushrooms are to be processed and a higher dry matter content is essential, carpophores from the 4th cropping flush are more suitable, while mushrooms to be sold directly to consumers are best from the 1st flush, as they were found to be most aromatic.

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ISTOTNE DLA AROMATU ZWIĄZKI LOTNE PIECZARKI *AGARICUS BISPORUS* W ZALEŻNOŚCI OD WARUNKÓW UPRAWY

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Dokonano analizy aromatu pod względem zawartości związków ośmiowęglowych (1-okten-3-ol, 3-oktanone, 3-oktanol, 2-okten-1-ol) w próbach dwóch odmian pieczarki *Agaricus bisporus* – KORONA 7 i EUROMYCEL 12. Pieczarkę uprawiano doświadczalnie w latach 2001, 2002, 2003, w nowoczesnej pieczarkarni, w sezonie zimowo-wiosennym. Owocniki zbierano w 4 rzutach i sortowano według wielkości kapelusza. Dominującym ilościowo związkiem we wszystkich próbach był 1-okten-3-ol, który występował w najwyższym stężeniu w rzucie I zbiorów (tab. 1–4). Analiza wariancji dla doświadczeń czynnikowych wykonana dla obu odmian w jednym cyklu uprawowym – 2001/2002 oraz dla jednej odmiany – KORONA 7 z dwóch cykli, wykazała wpływ odmiany pieczarki, rzutu plonowania i wielkości owocnika na zawartość suchej masy i związków lotnych zapachowych. Owocniki o mniejszej średnicy kapelusza charakteryzowały się wyższą zawartością suchej masy, a przeważnie także wyższą zawartością związków lotnych zapachowych (tab. 5–10). Natomiast wpływ rzutu plonowania był odmienny: najwyższą zawartość suchej masy stwierdzono w IV-tym rzucie, podczas gdy dla związków zapachowych w I-szym.